Please amend the application as follows: IN THE CLAIMS:

MARKED-UP VERSION OF THE AMENDED CLAIMS

1. (currently amended) Reaction chambers coated with native, synthetically or enzymatically prepared nucleic acids, wherein said coating is performed non-covalently with a calibrated nucleic acid and a carrier nucleic acid at the surface of the inner walls of reaction chambers which surface of the inner walls does not require any chemical nor biochemical modification prior to coating , which reaction chambers are storable without problems for a prolonged period of time with unchanged quality.

2. (previously presented) Reaction chambers according to claim 1, wherein they are comprised
of glass or plastic vessels or of glass capillaries , and wherein they are useable for kits.

3. (previously presented) Reaction chambers according to claim 1, wherein DNA, RNA,

synthetic equivalents of DNA and/or RNA, as well as dU-containing DNA

after calibration are used as calibrated nucleic acids.

4. (previously presented) Reaction chambers according to claim 1, further comprising

a) for dilution of DNA standards, a DNA solution is used comprising a nucleic acid compound having a minimum sequence homology to the nucleic acid compound to be analyzed, and

b) a tRNA solution is used for [the] dilution of the RNA standards.

5. (currently amended) Reaction chambers according to claim 1, ~~further comprising~~ wherein the carrier nucleic acid [[being]] is DNA of the lambda phage which is converted into readily soluble fragments of a mean length of about 1 - 2 kb by means of ultrasonic treatment, and wherein carrier nucleic acid is added to the calibrated nucleic acid, wherein the carrier nucleic acid generates an improved

adsorption during a lyphilization process, an increased stability of the calibrated nucleic acid in the reaction chambers, and [[serve]] is used in a solution for producing a thinning sequence out of the calibrated nucleic acid.

6. (previously presented) A method for the production of reaction chambers comprising directly aliquoting calibrated standard nucleic acids and added carrier nucleic acid into reaction chambers, and subsequently non-covalently adsorbing the calibrated standard nucleic acids and added carrier nucleic acids directly in the inner wall of the reaction chamber by means of freeze-drying or vacuum-centrifugating lyophilization.

7. (previously presented) Method according to claim 6, wherein plastic vessels or glass capillaries are coated.

8. (previously presented) Method according to claim 6 further comprising

using DNA, RNA, synthetic equivalents and/or RNA, as well as dU-containing DNA as nucleic acids.

9. (previously presented) Method according to claim 6 further comprising

a) for the dilution of DNA

standards, a DNA solution is used comprising a nucleic acid having a minimum sequence homology to the nucleic acid compound to be analyzed, and

b) a tRNA solution is used for the dilution of the RNA standards.

10. (previously presented) Method according to claim 6, wherein said carrier nucleic acid is DNA of a lambda phage which is converted into readily soluble fragments of a mean length of about 1-2 kb by means of ultrasonic treatment.

11. (previously presented) Method according to claim 6 further comprising

simultaneously coacting, if necessary, reaction chambers with a multitude (i.e. at least two) of application-specific calibrated nucleic acids of different cellular or organic origin, or originating from different species.

12. (previously presented) Method according to claim 6, wherein said coating is performed of at least 96 reaction chambers which are arranged in a microtiter format and which comprise at least 12x 8-well strips containing carrier nucleic acids and calibrated nucleic acids while an arbitrarily chosen concentration of each calibrated nucleic acid differs stepwise from well to well (highest concentration in A1-A12, lowest

concentration in H1-H12) in order to cover the entire concentration range of an analyte nucleic acid to be measured.

13. (previously presented) Method according to claim 6, wherein the coated reaction chambers are sealed and standing upright in an appropriate carrier box receiving at least 96 vessels.

14. (previously presented) Method according to claim 6, wherein apart from the calibrated standard nucleic acids, at least two oligonucleotides which are either 5'- and/or 3`- labeled with a fluorescent or non-fluorescent chromophore or unlabeled, the carrier nucleic acid and further components required for enzymatic nucleic acids amplification are contained in the reaction chambers in a lyophilized formulation, or at least two oligonucleotides, the carrier nucleic acid and further components required for enzymatic nucleic acids amplification are contained in separate vessels without said standard nucleic acids in a lyophilized formulation.

15. (previously presented) Method according to claim 6 further comprising using the reaction chambers coated with nucleic acids in test kits for the detection of selected nucleic acids in biological substances.

16. (previously presented) Method according to claim 15 further comprising

using test kits comprised of an octet strip of closed reaction chambers coated with eight different nucleic acid concentrations of at least two oligonucleotides, as well as one carrier nucleic acid and closed with a film / foil.

17. (previously presented) A method for producing reaction chambers comprising employing a chamber wherein calibrated standard nucleic acids and added carrier nucleic acids are directly aliquoted into the chamber, lyophilizing the calibrated standard nucleic acids and the added carrier nucleic acids by freeze-drying or vacuum-centrifugating; and non-covalently adsorbing directly the calibrated standard nucleic acids and the added carrier nucleic acids onto the inner wall of the chamber and thereby producing a reaction chamber.

18. (previously presented) The method according to claim 17 further comprising

non-covalently adsorbing directly the calibrated standard nucleic acids and the added carrier nucleic acids onto plastic vessels or glass capillaries.

19. (previously presented) The method according to claim 17 further comprising

employing DNA, RNA, synthetic equivalents and/or RNA, as well as dU containing DNA as calibrated standard nucleic acids.

20. (previously presented) The method according to claim 17 further comprising

employing a DNA solution comprising a nucleic acid having a minimum sequence homology to the nucleic acid compound to be analyzed for a dilution of DNA standards, and employing a tRNA solution for a dilution of RNA standards.

21. (previously presented) The method according to claim 17 further comprising

furnishing a carrier nucleic acid by employing a DNA of a lambda phage, and converting the DNA of the lambda phage into readily soluble fragments of a mean length of about 1 - 2 kb by means of ultrasonic treatment.

22. (previously presented) The method according to claim 17 further comprising simultaneously coating the reaction chamber with a multitude of application-specific calibrated nucleic acids of different cellular or organic origin, or originating from different species.

23. (previously presented) The method according to claim 17 further comprising

performing coating of at least 96 reaction chambers which are arranged in a microtiter format and which comprise at least 12x 8-well strips containing carrier nucleic acids and calibrated nucleic acids while an arbitrarily chosen concentration of each calibrated nucleic acid differs stepwise from well to well (highest concentration in A1-A12, lowest concentration in H1-H12) in order to cover the entire concentration range of an

24. (previously presented) The method according to claim 17 further comprising sealing coated reaction chambers; and standing the coated reaction chambers upright in an appropriate carrier box receiving at least 96 vessels.

25. (previously presented) The method according to claim 17 further comprising

employing at least two oligonucleotide primers or probes which are either 5`- and/or 3`- labeled with a fluorescent or non-fluorescent chromophore or unlabeled apart from the calibrated standard nucleic acids; containing the carrier nucleic acid and further components required for enzymatic nucleic acids amplification in the reaction chambers in a lyophilized formulation, or at least two oligonucleotide primers or probes; and containing the carrier nucleic acid and further components required for enzymatic nucleic acids amplification in separate vessels without said standard nucleic acids in a lyophilized formulation.

26. (previously presented) The method according to claim 17 further comprising forming a test kit for a detection of selected nucleic acids in biological substances with reaction chamber.

27. (previously presented) The method according to claim 17 further comprising

forming a test kit comprising an octet strip of closed reaction chambers coated with eight different nucleic acid concentrations of at least two oligonucleotides, as well as one carrier nucleic acid and closed with a film / foil.

28. (previously presented) The method according to claim 17 further comprising

forming a test kit comprising a strip of eight reaction vessels coated with eight different amounts of at least one calibrated standard nucleic acid, carrier nucleic acid and at least two oligonucleotides and which is sealed with an appropriate self-adhesive foil.

29. (previously presented) A reaction chamber obtained by employing a method for

producing reaction chambers comprising employing a chamber wherein calibrated standard nucleic acids and added carrier nucleic acids are directly aliquoted into the chamber; lyophilizing the calibrated standard nucleic acids and the added carrier nucleic acids by freeze-drying or vacuum-centrifugating; and non-covalently

adsorbing directly the calibrated standard nucleic acids and the added carrier nucleic acids onto the inner wall of the chamber and thereby producing a reaction chamber , wherein the reaction chamber is suitable to be stored at room temperature for a period longer than a year without loss of quality.

30. (previously presented) A coated reaction chamber comprising

a reaction chamber;

an inner wall of the reaction chamber;

a standard coating disposed on the inner wall of the reaction chamber immediately with native, synthetically or enzymatically prepared standard nucleic acids, wherein the standard coating is performed non-covalently with a mixture of calibrated nucleic acids, the standard nucleic acids and carrier nucleic acids at the surface of the inner walls of the reaction chambers, which inner walls of the reaction chambers do not require any chemical nor biochemical modification prior to the coating.

31. (previously presented) The coated reaction chamber according to claim 30, wherein the reaction chamber comprises glass or plastic vessels or glass capillaries.

32. (previously presented) The coated reaction chamber according to claim 30, wherein the standard nucleic acids include a member of the group consisting of DNA, RNA, synthetic equivalents of DNA and/or RNA, as well as dU-containing DNA .

33. (previously presented) The coated reaction chamber according to claim 30, wherein the standard nucleic acids comprise

a) a DNA solution is used comprising a minimum

sequence homology to the nucleic acid compound to be analyzed for a dilution of DNA standards, and

b) a tRNA solution is used for a dilution of the RNA standards.

34. (previously presented) The coated reaction chamber according to claim 30, wherein said carrier nucleic acid is a DNA of the lambda phage, which carrier nucleic acid is converted into readily soluble

fragments of a mean length of about 1 - 2 kb by means of ultrasonic treatment.

35. (new) A method for producing coated reaction chambers comprising the steps of:

preparing an adsorb able standard nucleic acid;

purifying the adsorb able standard nucleic acid;

determining a precise concentration of the adsorb able standard nucleic acid by means of high performance liquid chromatography (HPLC) thereby furnishing a calibrated nucleic acid;

preparing a dilution member from the calibrated nucleic acid with an addition of defined quantities of a carrier nucleic acid;

employing a chamber;

directly aliquoting the dilution member into the chamber;

lyophilizing the calibrated nucleic acid and the added carrier nucleic acid of the dilution member by freeze-drying or vacuum-centrifugating; and

non-covalently adsorbing directly the calibrated nucleic acid and the added carrier nucleic acid of the dilution member onto an inner wall

of the chamber and thereby producing a coated reaction chamber suitable for enzymatic amplification reactions.

36. (new) The method according to claim 35 further comprising the steps of

using these coated reaction chambers as standards;

transforming the coated reaction chambers into a form imperishable for prolonged periods of time without impairments of quality.

37. (new) The method according to claim 35 further comprising the steps of furnishing an automated quantitative measurement of smallest amounts of analyte nucleic acids in diverse biological materials in conjunction with a previous enzymatic amplification;

transforming nucleic acids into a form appropriate for the standardization of quantitative enzymatic amplification reactions;

producing nucleic acid-coated, so-called "ready-to-use" standard reaction chambers, which are of simple use, which are storable without problems for a prolonged period of time with unchanged

quality, and which may be used as components for test kits, and hence better comply with the requirements of routine diagnostic laboratories employing automated analyses;

wherein the standard nucleic acids are single-stranded or double-stranded DNA or RNA or synthetic equivalents of DNA and RNA, as well as DNA, the native deoxythymidine (dT) bases thereof being completely or partially substituted by deoxyuracil (dU);

preparing standard nucleic acid by means of polymerase chain reaction (PCR) with the use of specific primer oligonucleotides.

38. (new) The method according to claim 35 further comprising the steps of

making the standard nucleic acid with native amplification products prepared enzymically, or synthetic nucleic acids are used, where the nucleotide sequence of the standard nucleic acid is homologous to a sequence to be determined, or is identical or characterized by one or more point mutations, deletions or insertions, which preferably lie outside of a primer or probe bonding point; or

39. (new) The method according to claim 35 further comprising the steps of

preparing DNA standards by means of enzymatic amplification of target sequences, preferably by polymerase chain reaction (PCR);

cleaning DNA by means of agarose gel electrophoresis and subsequent extraction of the standard nucleic acid from the separating gel;

40. (new) The method according to claim 35 further comprising the steps of

preparing RNA fragments by means of *in vitro* RNA synthesis with the use of RNA polymerases.

subjecting the prepared nucleic acid fragments subsequently to a purification procedure;

extracting *in vitro*-prepared RNA from the *in vitro* synthesis assay;

performing a precise measurement of the concentration of the purified nucleic acid product by means of HPLC;

41. (new) The method according to claim 35 further comprising the steps of

preparing a dilution series subsequently of the calibrated standard nucleic acid;

diluting DNA standards by employing a DNA solution, wherein the DNA solution is prepared by transforming the DNA of the lambda phage by means of an ultrasonic treatment into fragments of about 1 - 2 kilobases (kb) for inducing an improved adsorption during the lyophilization process and to contribute to an increased durability of the standard nucleic acid in the reaction chamber.;

42. (new) The method according to claim 35 further comprising the steps of

using the lambda DNA unmodified, or

using E.coli tRNA; or

using a transport RNA (tRNA) solution for the dilution of RNA standards,

43. (new) The method according to claim 35 further comprising the steps of

preparing various standard dilutions for the quantification of a measurement parameter, , wherein the standard dilutions allow for the measurement of the entire physiological or technological concentration range of the analyte to be measured;

using aliquots of the standard dilutions for coating those reaction chambers, wherein the enzymatic nucleic acid amplifications necessary for the establishment of an calibration graph, are to take place.

coating eight separate reaction chambers with eight different standard dilutions (so-called octet strip).

mildly drying aliquots of respective standard nucleic acid dilution supplemented by the carrier nucleic acid directly in the reaction chambers used;

performing lyophilization by means of a vacuum centrifuge or a freeze-drier;

drying for obtaining a superheating-free product;

drying with the use of microwaves.

44. (new) The coated reaction chamber according to claim 30,

wherein the coated reaction chambers produced are characterized in that the adsorbed nucleic acid standards adhere so fixedly on the inner surface of the reaction chamber used for coating, that problem-free shipment via mail may is guaranteed;

wherein the coated reaction chambers are standing upright in a suitable carrier box receiving at least 96 vessels, the coated reaction chambers are closed with a self-adhesive film, so as to avoid contamination with foreign nucleic acids during storage and transport.;

wherein in each case one octet strip is closed with a film / foil, hence a strip containing 8 different concentrations of the nucleic acids used for coating, is designated as a "ZeptoStrip".

45. (new) The coated reaction chamber according to claim 30,

wherein said reaction chambers, apart from the calibrated nucleic acids, further comprise at least two specific marked or unmarked oligonucleotides acting as primers or probes, as well as further reaction components required for the enzymatic amplification, are contained in a lyophilized form;

wherein reaction chambers used solely contain in a lyophilized form these specific oligonucleotides acting as primers or probes, the carrier nucleic

acid, and further reaction components required for the enzymatic amplification.

46. (new) The coated reaction chamber according to claim 45,

wherein the coated reaction chamber is part of a test kit including at least one "ZeptoStrip", with at least two oligonucleotides and one carrier nucleic acid.

REMARKS

Claims 1 through 34 continue to be in the case.

New claims 35 to are being submitted.

New claim 35 is based on claim 17 and the specification, page 4, lines 1 through 5.

New claim 36 is based on the specification, page 4, lines 6 through 9.

New claim 37 is based on the specification, page 4, line 15 through page 5, line1.

New claim 38 is based on the specification, page 5, line1 through page 5, line15.

New claim 39 is based on the specification, page 5, line1 through page 5, line15.

New claim 40 is based on the specification, page 5, line1 through page 5, line15.

New claim 41 is based on the specification, page 5, line 15 through page 5, line23.

New claim 42 is based on the specification, page 5, line 23 through page 5, line27.

New claim 43 is based on the specification, page 5, line 29 through page 6, line11.

New claim 44 is based on the specification, page 6, line12 through page 7, line 4.

New claim 45 is based on the specification, page 7, line 6 through page 7, line11.

New claim 46 is based on the specification, page 7, line 12 through page 7, line13.

The Office Action mailed on February 23, 2004 states that this is a ***Continued Examination Under 37 CFR 1.114.***

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on September 5, 2003 has been entered.

Applicant appreciates the continued examination.

2. Applicant's amendment filed July 15, 2003 has been entered. The amendment overcame the following: objections to claims 1, 6, 14, 17, 25, 28 and 29 and rejection of claims 1-29 under 35 U.S.C. 112, second paragraph. Rejection of claims 1-5 and 29 under 35 U.S.C. 102(b) over Day et al. is maintained for reasons given in the "Response to Arguments" section.

3. Claims 1-34 are pending and will be considered.

The Office Action refers to Response to Arguments.

4. Applicant's arguments filed July 15, 2003 have been fully considered but they are not persuasive.

Regarding rejection of claims 1-5 and 29 under 35 U.S.C. 102(b) over Day et al., Applicants argues that addition of a limitation "which reaction chambers are storable without problems for a prolonged period of time with unchanged quality" to claim 1 and addition of a limitation "wherein the reaction chamber is suitable to be stored at room temperature for a period longer than a year without loss of quality" to claim 29 overcome the rejection, since the plates of Day et al. withstand the conditions of postal transport.

The new limitations are not structural limitations, i.e., they do not introduce any characteristics into the product which distinguishes the product from the prior art. Also,

since the plates of Day et al. contain the same elements as the claimed reaction chambers of the instant application, they are expected to exhibit the same properties, namely, to be storable without problems for prolonged periods of time and to be storable at room temperature.

The rejection is maintained.

Applicants agree that the are not directly structural limitations. Nevertheless, they are important features of the coated reaction chambers.

The Office Action is correct in asserting that the determination of purpose "which reaction chambers are storable without problems for a prolonged period of time with unchanged quality" is no structural feature of claim 1.

However, the determination of purpose is to be considered a valuable, surprising property of the coated reaction chambers according to claim 1. This property was achieved by the procedure according to the present Invention, namely by the coating of bio chemically not modified surfaces of the inner wall of the reaction chambers with calibrated standard nucleic acids under the addition of defined quantities of carrier nucleic acids in a noncovalent way. An equivalent German Patent has issued for the subject

matter, since it was not predictable that these surprising, valuable properties could be achieved with the described method.

The Office Action refers to Claim Rejections - 35 USC § 112.

6. Claim 5 stands rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 is indefinite because of the limitation "serve for producing a thinning sequence out of the calibrated nucleic acid". It is not clear what it means to "produce a thinning sequence out of nucleic acid".

Claim 5 is being amended to obviate the rejection.

The Office Action refers to Claim Interpretation.

7. The term "reaction chamber" is interpreted as any container.

8. The terms "calibrated nucleic acid", "carrier nucleic acid" and "standard nucleic acid" are interpreted as any nucleic acid, since they were not defined in the specification.

A basic error of the Office Action is present at the point "Claim Interpretation". According to the opinion of the Office Action any arbitrary nucleic acid is to be understood as a " calibrated nucleic acid", " carrier nucleic acid", and " standard nucleic acid", since allegedly the definitions of these concepts were not explained in the description.

This assumption of the Office Action is incorrect. It is stated on page 1 of the description (lines 25 to 31) that standard nucleic acids, are nucleic acids of precisely defined concentration and that the production of these standards is known to a person of ordinary skill in the art. A standard nucleic acid consequently is no arbitrary nucleic acid. A standard nucleic acid is the precondition for quantitative determination of nucleic acids of unknown concentration. The equalization of "standard nucleic acids" with " any arbitrary nucleic acid" as propounded in the Office Action contradicts the assumptions of a person of ordinary skill in the art. It is a clear difference, if any nucleic acids or standard nucleic acids are entered into the reaction chambers. The reference Day et al. (Biotechniques 18 (1995), 981-984) describes a qualitative determination of nucleic acids. No standard nucleic acids are necessary for the procedure of the reference Day et al.. The present Invention is thereby clearly distinguished from the publication

of Day et al. under the precondition that the distinction is made between any nucleic acid" and "standard nucleic acid".

It is not a purpose of the present Invention to coat any reaction chambers with arbitrary nucleic acids but to furnish reaction chambers for quantitative PCR.

Applicant respectfully submits the following possibilities of definition of terms:

A. The term " nucleic acid " means any nucleic acid.

B. The term "standard nucleic acid" means any synthetic, recombinant, cloned or in vitro synthesized DNA or RNA with mono-specificity for a given target DNA or RNA, which is susceptible to any kind of specific calibration by analytical methods such as HPLC and which is suited to serve in a proper calibrated state as a positive control or quantification control in a subsequent detection procedure, preferably a nucleic acid amplification technology. Said standard nucleic acid is manufactured in a concentrated master batch containing about 10E10 - 10E11 copies per µl of preparation

and is finally used in a highly diluted and stabilized state containing between 10-10,000,000 copies per reaction only.

C. The term " calibration of nucleic acid" means exact determination of the concentration or copy number of standard nucleic acid preparations by means of bioanalytical methods, e.g. biochemical, spectrophotometrical, or fluorescence detection protocols. Calibration of a defined, highly purified nucleic acid is the prerequisite for manufacture of a standard nucleic acid lot to be used as control which always contains an fixed copy number in a given vessel.

4. The term "Carrier nucleic acid" means any synthetic or physiologic DNA or RNA preferably submitted to physical cleavage into smaller debris e.g. by sonication, whereas the base sequence of the carrier nucleic acid is completely different from the sequence of the target nucleic acid in order to avoid any interference between target detection and carrier nucleic acid. Carrier nucleic acids are as long carriers as they do not give any signal after running a subsequent specific detection protocol for a given target.

Standard nucleic acids represent a selection from the larger region of nucleic acids. Since no standard nucleic acids are described in the reference Day et al. or sensibly captured and since the person of ordinary skill in the art can clearly distinguish between arbitrary nucleic acids and standard nucleic acids, therefore the feature "standard nucleic acids" of claim 1 of the present application is novel relative to the reference Day et al.

In addition, the equalization of the term "carrier nucleic acid" with "any nucleic acid" is incorrect. Page 2, lines 9 through 14 of the description states that a carrier nucleic acid exhibits if possible a minimum sequence homology relative to the nucleic acid sequence to be detected. Corresponding references have been recited and made available to the United States Patent and Trademark Office. A carrier nucleic acid therefore is no arbitrary nucleic acid. The equalization of "carrier nucleic acid" with "any nucleic acid" contradicts the concepts of a person of ordinary skill in the art. It is a clear difference, if any nucleic acids or carrier nucleic acids (that is nucleic acids with if possible minimum sequence homology relative

to the nucleic acid sequence to be detected) can be placed into the reaction chambers. The reference Day et al. does not teach any carrier nucleic acids.

Precisely the method according to the present Invention leads to the valuable properties of the coated reaction chambers, namely the stability of the nucleic acid standards, which stability is superior to that of the state-of-the-art, such that these nucleic acid standards can be employed as standards, as defined coated reaction chambers and be transformed into a form, which form is stable over long time periods without that interferences of the quality occur. This is a surprising, not predictable result of the Invention method.

9. The following rejection is based on the product claimed in claims 1, 29 and 30, which is "Reaction chambers coated with native, synthetically or enzymatically prepared nucleic acids", irrespective of the way in which they were obtained (see MPEP 2113 and 2114). Further, the limitations "storable without problems for a prolonged period of time with unchanged quality" (claim 1) and "suitable to be stored at room temperature for a period longer than a year without loss of quality" (claim 29) refer to the properties of the reaction chambers, not their structural limitations. The limitations "useable for kits"

(claim 2), use of dilution solutions (claims 4 and 33) are intended use limitations, which, again, do not impose structural constraints on the product (see MPEP 2114).

Applicant agrees that a use for kits or a use in a dilution sequence is not a direct structural limitation.

The Office Action refers to Claim Rejections - 35 USC § 102.

10. Claims 1-4. 29 and 30-33 stand rejected under 35 U.S.C. 102(b) as being anticipated by Day et al. (Biotechniques, vol. 18, pp. 981-984, 1995; cited in the previous office action).

The present Invention can be explained as follows:

The quantitative determination of nucleic acids is of concern. The sequences of the nucleic acids (qualitative determination) to be determined are known. The concentrations of the nucleic acids are unknown. It is possible to establish a calibration curve for determining the concentrations of the nucleic acids, such as for example in photometric measurements. The concentration of the nucleic acid to be determined is to the concluded based on this calibration curve. It is a precondition for the establishment of a calibration curve that the concentrations of the materials, for which the

calibration curve is established, are known precisely. Inaccuracies lead to wrong results. The inaccuracies can occur based on the fact that solutions for production of the calibration curve change their concentration, for example based on lysis. For this reason it has been proposed in the state-of-the-art to prepare the solutions daily anew. According to experience in this connection also occur errors, for example pipetting errors or thinning errors. As mentioned in the office action, it has been proposed by Koehler et al. for stabilizing the solutions to add a carrier nucleic acid to the solutions. Improvements showed up here in fact, however the result was still unsatisfactory. However, the teaching of Koehler et al is directed to solutions. It was a purpose of the present Invention to find possibilities to maintain the concentration of the nucleic acids, with which nucleic acids calibration curves can the established, constant over a longer time period. Of concern here are dried coated reaction chambers and not the solutions of concern in Koehler et al.

In fact, Day et al. have proposed to coat vessels with nucleic acids, however only for the qualitative diagnostic of nucleic acids. The term standard DNA or an equivalent thereof was nowhere employed in the whole document of Day et al.. The use of standard nucleic acids is also not

necessary considering the purposes of the reference Day et al., since a qualitative diagnostic of nucleic acids is the concern of the reference Day et al.. Consequently, the molecule precise storage stability of nucleic acids is only of subordinated importance for the reference Day et al., since all reactants are employed in a stochiometric excess. For this reason the reference Day et al. in contrast to the present Invention fails to furnish any experimental proof for proven storage stability. Possible processing concentration between the starting material (that is prior to drying) and final product (that is after drying) are not verified by the reference Day et al.. The undefined drying conditions (drying over several hours at room temperature, p. 982) employed by the reference Day et al. would with certainty lead to a partial lysis of the extremely low concentrated standard DNA/RNA molecules, if hypothetically the method of the reference Day et al. would be transferred to the (highly thinned) standard nucleïc acids. In addition it is to be considered that not only alone the absorption technology are extremely critical parameters, but also be complete desorption of the standard nucleic acids are extremely critical parameters, wherein both these extremely critical parameters together were completely removed as such for the first time by the present Invention.

The reference Day et al. fails to teach or to suggest to resolve the problem of the lack of stability of standard nucleic acids by lyophilization.

Up to now it has not been proposed to coat vessels or other reaction chambers with highly thinned nucleic acids of precisely defined concentration, where the thinning agent contains a carrier nucleic acid and wherein the carrier nucleic acid serves simultaneously as a stabilizer. In view of the reasons presented above, the reference Day et al. in no way suggests such coating with highly thinned nucleic acids of a precise concentration.

The office action propounded that the present Invention is obvious in view of the combination of the teachings of the references Koehler et al. and Day et al.. To make such determination it is necessary to understand the features of the present Invention. The reference Day et al. fails to teach that standard nucleic acids (which are highly thinned nucleic acids of known concentration) can be stabilized by coating on a chamber. The opinion of the office action that such a stabilization would logically follow, in case the teachings of the references Day et al. and Koehler et al. would be combined, is not based on the teachings and suggestions of the two references, but

instead is sheer hindsight in view of the present Invention and consequently not a permissible consideration.

The quality of the standard nucleic acids is based in particular on the addition of the carrier nucleic acid, which simultaneously serves the purpose of thinning, and on the careful drying.

The distinction of the present Invention from the reference Day et al. is based on the employment of highly thinned nucleic acids with a precisely known concentrations, which are specific for the nucleic acids to be determined and wherein the nucleic acids are thinned with carrier nucleic acids for obtaining a calibration curve.

The present Invention for the first time establishes a method to provide stable storage of standard nucleic acids. There was no known method in the arts of stabilizing nucleic acids. Thereby it has become possible to furnish reaction chambers (for example vessels), wherein the reaction chambers contain the specific nucleic acids of precisely known concentration as well as carrier nucleic acids in lyophilized form necessary for the establishment of a calibration curve.

Applicant further submits that the long-term stability of the standard nucleic acids of the present Invention is based on a synergistic effect.

Even though it was known that the addition of carrier nucleic acids renders standard nucleic acids more stable in solution, nevertheless based on the careful drying of the standard nucleic acid/carrier nucleic acid mixtures there has been achieved a stability of the standard nucleic acids which has surpassed all expectations. Now these reaction chambers can be packaged, labeled, shipped and stored without that changes in concentration occur. Analytical laboratories do not have any longer to prepare daily new standard nucleic acids (together with the therewith associated errors), but the analytical laboratories need only open the packaged reaction chambers and can immediately measure the concentration of the nucleic acids to be determined. The present Invention thereby furnishes for the first time "ready to use of solutions" for determining concentrations of nucleic acids.

According to the Office Action, Day et al. teach 96-well plates coated with DNA templates which were dried in the wells. The plates can then be used for setting up PCR reactions. Alternatively, PCR primers are distributed into the wells and dried there. In both cases, adherence of the dried DNA to the walls of the wells is non-covalent, since

both dried template and dried primers function in subsequent PCR reactions (page 381-383).

Applicant respectfully traverses.

The arguments relating to the reference of Day et al., Biotechniques 18 (1995), 981-984 have already been controverted previously by the inventors and are again summarized as follows:

The reference Day et al. is focused on qualitative applications of the method whereas the present invention is directed to quantitative analytical use.

A molecule-accurate stabilization of standard nucleic acids is neither aim of the publication Day et al. nor shown in the results, but are the purpose of the present invention.

A complete desorption of the dried-down genomic DNA is neither aim nor teaching of the reference Day et al. but, in contrast, represents a purpose the present invention

No synthetic and exactly calibrated standards are suggested or taught by the Day et al. reference.

The preparations of the reference day et al. are performed by non-standarized protocols (dried on the lab bench!) whereas the present

invention and application uses and claims a standardized lyophilization protocol.

No distinct carrier nucleic acids are mentioned or added by the reference Day.et al..

A simultaneous coating with standards and reaction components as primers, probes, dNTPs are not suggested in contrast to applicants' disclosure and claims.

11. Claims 1, 3, 4, 6, 8, 11, 14, 15, 17, 19, 22, 25, 26, 29, 30, 32 and 33 stand rejected under 35 U.S.C. 102(b) as being anticipated by Klatser et al. (J. Clin. Microbiol., vol. 36, pp. 1798-1800, June 1998).

Regarding claims 1, 3, 4, 29, 30, 32 and 33, Klatser et al. teach containers with DNA primers which were non-covalently adsorbed onto the surface by freeze-drying (page 1798, third paragraph; page 1799, second paragraph).

Regarding claims 6 and 17, Klatser et al. teach a method for the production of reaction chambers, the method comprising directly aliquoting calibrated standard nucleic acids and added carrier nucleic acid into reaction chambers and subsequently non-covalently adsorbing the calibrated standard nucleic acids and added carrier nucleic acids directly in the inner wall of the reaction chamber by means of freeze-drying or

vacuum-centrifugating lyophilization (Klatser et al. teach directly adsorbing DNA primers onto container walls by lyophilization of batches of PCR mixes, comprising PCR primers (page 1798, third paragraph). Klatser et al. do not specifically teach a container, but since the samples were lyophilized, they had to be placed in a container, therefore, inherently, Klatser et al. teach the limitations of these claims).).

Regarding claims 8 and 19, Klatser et al. teach using DNA primers (page 1798, third paragraph).

Regarding claims 11 and 22, Klatser et al. teach primers for detection of two different Mycobacterium tuberculosis genes, IS61 10 and 16S rRNA (page 1798, second paragraph).

Regarding claims 14 and 25, Klatser et al. teach lyophilizating PCR reaction mix comprising primers, DNA polymerase, dNTPs and uracil-DNA-glycosylase (page 1798, third paragraph).

Regarding claims 14, 15 and 25, Klatser et al. teach forming a kit for the detection of Mycobacterium tuberculosis (page 1799, second paragraph).

Applicants respectfully disagree with the rejection.

The publication of Klatser et al. (J. Clin.Microbiol. 1998) is similarly unsuitable as an applied reference for rejecting applicants' claims as is the reference Day et al., since also the reference Klatser et al. does not describe any standard nucleic acids. The opinion of the office action that this

reference Klatser et al. teaches calibrated standard nucleic acids and carrier nucleic acids is incorrect. The concept " standard nucleic acid" does not appear at all in the reference Klatser et al..

Here again appears that the Office Action in error equates the concept standard nucleic acids with any arbitrary nucleic acids.

Furthermore, the arguments of Klatser et al. (J. Clin. Microbiol. 1998) are not applicable to suggest or teach applicants' claims for the following reasons:

The referénce Klatser et al. reports the need of addition of trehalose for optimal freece-drying. The addition of this sugar as a stabilizing agent is known at least since 1989 and several patents have issued regarding to this addition. The claims of the present application do not call for any addition of trehalose.

In contrast to the present invention, the reference Klatser et al. teaches that batches of reagents are lyophilised and not aliquots, which aliquots are required to run one ready-to-use test as shown in the present invention and required in the claims of the present application.

The reference Klatser et al. describes the stabilization of complete reaction mixtures including enzymes. Stabilization of enzymes is not the purpose of the present invention.

The stabilization of standards is never mentioned in the reference Klatser et al. Purified DNA is only used by the reference Klatser et al. to check the functionality and performance of the lyophilised master reagent batch.

The Office Action refers to Claim Rejections - 35 USC § 103.

12. Claims 5 and 34 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Day et al. (Biotechniques, vol. 18, pp. 981-984, 1995; cited in the previous office action), Koehler et al. (Biotechniques, vol. 23, pp. 722-726, 1997; cited in the IDS) and Barany et al. (U.S. patent No. 5,494,810).

A) Day et al. do not teach carrier nucleic acid being k DNA.

B) Koehler et al. teach addition of k DNA digested with HindIII as a carrier to stabilize standard DNA in solution (page 724, second paragraph; page 725, second paragraph). Koehler et al. do not teach sonicated k DNA.

C) Barany et al. teach using sonicated salmon sperm DNA as a carrier (col. 34, lines 29, 30).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time of

the invention to have used k DNA as a carrier in the formation of plates of Day et al. The motivation to do so, provided by Koehler et al., would have been that presence of carrier DNA stabilized DNA in dilute solution and prevented non-specific binding of DNA to the tubes (page 724, second paragraph) and diminished variability in the amplification reactions using the competitor in solution (page 725, second paragraph). The motivation to sonicate the k DNA of Koehler et al., provided by Barany et al., would have been that the sonicated DNA provided no background in amplification reactions (col. 36, lines 21-23).

Claims 5 and 34 of the present application are stated to be obvious according to the office Action based on the references of Day et al., Koehler et al. and Barany et al.

Applicant respectfully disagrees. The reference Barany et al. does not teach the addition of carrier-DNA for stabilization purposes, but as a stop reagent.

The Office Action is of the opinion that it is obvious in first view to employ lambda-DNA as a carrier (as described by Koehler et al.) in the plates of Day et al.. According to the Office Action a person of ordinary skill would be led to the claimed Invention, if the teachings of Koehler et al. and of Day et al. would be combined.

As already described above, the reference Day et al. is not concerned with the quantitative determination of DNA, but with a qualitative analysis. It is therefore questionable if the teaching of Day et al. can be at all employed for the determination of obviousness, since qualitative analysis and quantitative analysis are clearly different fields.

Even if one would combine the teachings of the references Day et al. and Koehler et al., it could not be predicted, that the reaction chambers coated in this way with nucleic acids could be produced with the high stability. In fact, the reference Koehler et al. describes the addition of carrier-DNA as a stabilization agent for DNA-standards, however it is stated that the same time that this does not resolve the stability problem completely. Looking to figure 2 on page 7 under 25 of the mentioned paper (Koehler et al.) clearly shows that although the stability is improved, the effective loss of standards molecules in solution is still going along despite addition of carrier DNA as indicated by the shift of the crossing points with the Y=0 axis to the ride. The solution of this problem is firstly achieved by using Stabilizor DNA-coated strips!).

Applicants urge that no basis is given in the Office Action, as to why a relative stability of some solutions should be predictive for a stability of coatings.

It is therefore not correct to say that the combination of the teachings of the references Day et al. and Koehler et al. leads to the subject matter of the present application..

Applicant urges that a combination of the references of Koehler et al. with Day et al. would not lead to any coating whatsoever, but to a solution in view of the positive stability assertions of Koehler et al. for solutions.

Based on a combination of the references Day et al. and Koehler et al. it could not be expected that the addition of carrier DNA would have the same effect as was described for solutions by the reference Koehler et al.. No person of ordinary skill in the art could have predicted that a coating of the inner walls of the reaction chambers with calibrated standard-DNA and carrier-DNA would result in a far superior stability in particular as compared to the stability described in the reference Kohler et al. for solutions.

Consequently a synergistic effect occurred, where the synergistic effect was surprising and wherein the synergistic effect for the first time effectively resolves a previously unresolved problem. The coated reaction chambers according to the present Invention furnish for the first time "ready to use" nucleic acids standards, which nucleic acid standards have proven to be simple in the application, which nucleic acid standards can be stored over long time periods with uniformly remaining quality and which nucleic acid standards can be employed as components of test kits, and which nucleic acid standards better meet the requirements of routine diagnostic laboratories in particular in view of automatic analysis.

The present Invention has more achieved as could be expected by the combination of the teachings of Day et al. and Koehler et al.. This fact meets the requirement for an inventive step. Consequently the present invention is not obvious over the references recited.

The claim rejections stated under point 12 of the Office Action are further traversed for the following additional reasons:

It is correct that Koehler et al. (where the inventor Koehler is identical with the inventor of the present invention carrying the same name) teach

addition of λ-DNA to stabilize standards in solution. But looking to figure 2 on page 725 of the mentioned reference paper clearly shows that although the stability is improved the effective loss of standards molecules in solution is still going on despite addition of carrier DNA as indicated by the shift of the crossing points with the Y=0 axis to the right. The solution of this problem is firstly achieved by using stabilized DNA-coated strips!

The reason of the citation of the reference Barany et al. (US 5,494,810) is not understood since the reference Barany et al. introduces DNA not as a carrier to stabilize reagents but clearly indicates the use of sonicated salmon sperm DNA as a stop reagent to terminate an enzymatic amplification reaction called LCR. I, unfortunately, do not see any relation to the present invention.

13. Claims 2, 7, 12, 13, 16, 18, 23, 24, 27 and 28 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Klatser et al. (J. Clin. Microbiol., vol. 36, pp. 1798-1800, June 1998), Cottingham (U.S. Patent No. 5,948,673), Irvine et al. (U.S. Patent No. 6,300,056) and Longiaru et al. (EP 0 420 260 A2).

A) Klatser et al. teach lyophilization of PCR reaction mixes, but do not specifically teach plastic or glass containers, 96 reaction chambers or different concentrations of aliquoted nucleic acids.

B) Regarding claims 2, 7 and 18, Cottingham teaches a DNA card comprising dried nucleic acid amplification reagents in the wells of sample chambers which are formed from plastic (col. 3, lines 45-48; col. 7, lines 55-64).

Regarding claims 12, 16, 23, 27 and 28, Cottingham teaches a DNA card comprising 64 identical sample cells, arranged in eigth rows and eight columns (col. 6, lines 19-25). The wells are sealed with a flexible, pressure sensitive material (col. 4, lines 5-10). The sealing strips cover one octet strip of the plate, to define segments which can be used individually (col. 6, lines 27-40).

C) Cottingham does not teach 96 reaction chambers or different concentrations of aliquoted nucleic acids.

D) Regarding claims 12, 13, 16, 23, 27 and 28, Longiaru et al. teach preparation of microplates with capture probes for quantitation of amplification reaction products. The known amounts (25 ng) of probes are non-covalently bound to the wells of either a 96-well plate or to strips of 12 tubes which fit into strip holders in a microtiter plate format, and the plates are sealed (page 6, lines 26-46).

E) Longiaru et al. do not teach different concentrations of probes.

F) Irvine et al. teach quantitation of HIV DNA by amplification of sample containing the HIV DNA on a microplate, the wells of which contain known amounts of HIV DNA in the range of 10 to 200 tmoles (1 tmole = 602 molecules), and preparing standard curve of the DNA concentration (col. 13, lines 17-50).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used multiple reaction chambers, such as wells on a microplate of Longiaru et al., and multiple concentrations of nucleic acid of Irvine et al., in the method of formation of reaction chambers of Klatser et al. The motivation to do so, provided by Cottingham, would have been that multiple well format can be conveniently handled by clinical personnel and all reagents for both DNA amplification and detection are provided within the device (col. 2, lines 45-49. 55-61). The motivation to do so, provided by Irvine et al., would have been that having a set of standard nucleic acids provided means for determining the concentration of HIV DNA down to 50 tmoles (= about 30,000 molecules) (col. 14, lines 5-17).

The claim rejections stated under point 13 of the Office Action are traversed for the following reasons:

Applicant repeats the points made in connection with point 11 of the Office Action.

The reference Cottingham (US 5,948,673) teaches dried nucleic acids as amplification reagents. Again, the reference Cottingham teaches no calibrated standards of exactly known concentration are mentioned within the described method.

The reference Longiaru et al (EP 0420260A2) describes oligonucleotides used for capturing nucleic acids. In contrast to the present invention these probes are, although not covalently bound to the surface of the plate, not desorbable from the well. If this would not be case the method of Longiaru would never function. In contrast, the present invention requires just this desorption to allow that the introduces standards are completely desorbed and, therefore, completely available as a target to the subsequent amplification process.

The reference Irvine only reports a dilution series of a standard but no stabilization of the highly diluted standards which is the purpose of the present invention. The manufacture of dilution series which is very frequently performed in diagnostic labs is not taught or suggested by the reference Irvine.

14. Claims 5, 10, 21 and 34 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Klatser et al. (J. Clin. Microbiol., vol. 36, pp. 1798-1800, June 1998), Koehler et al. (Biotechniques, vol. 23, pp. 722-726, 1997; cited in the IDS) and Barany et al. (U.S. patent No. 5,494,810).

A) Klatser et al. do not teach carrier nucleic acid being X DNA.

B) Koehler et al. teach addition of X, DNA digested with HindIII as a carrier to stabilize standard DNA in solution (page 724, second paragraph; page 725, second paragraph). Koehler et al. do not teach sonicated k, DNA.

C) Barany et al. teach using sonicated salmon sperm DNA as a carrier (col. 34, lines 29, 30).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used X, DNA as a carrier in the formation of plates of Klatser et al. The motivation to do so, provided by Koehler et al., would have been that presence of carrier DNA stabilized DNA in dilute solution and prevented non-specific binding of DNA to the tubes (page 724, second paragraph) and diminished variability in the amplification reactions using the competitor in solution (page 725, second paragraph). The motivation to sonicate the k, DNA of Koehler et al., provided by Barany et al., would have been that the sonicated DNA provided no background in amplification reactions (col. 36, lines 21-23).

Applicant respectfully traverses these rejections under point 14 based on the responses set forth above in connection with points 11 and 12 of the Office Action.

15. Claims 9 and 20 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Klatser et

al. (J. Clin. Microbiol., vol. 36, pp. 1798-1800, June 1998), Koehler et al. (Biotechniques, vol. 23, pp. 722-726, 1997; cited in the IDS) and Miyamura et al. (U.S. Patent No. 5,747,241).

A) Klatser et al. do not teach dilution of DNA standards using a DNA solution having a minimum sequence homology to the nucleic acid being analyzed, or dilution of RNA standards using tRNA solution.

B) Koehler et al. teach addition of k DNA digested with HindIII as a carrier to stabilize standard DNA in solution (page 724, second paragraph; page 725, second paragraph). Koehler et al. do not teach using tRNA.

C) Miyamura et al. teach adding tRNA to a serum sample which contains HCV RNA (col. 2, lines 63-67; col. 3, lines 1-9).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used k DNA of Koehler et al. as a carrier in the formation of plates of Klatser et al. The motivation to do so, provided by Koehler et al., would have been that presence of carrier DNA stabilized DNA in dilute solution and prevented non-specific binding of DNA to the tubes (page 724, second paragraph) and diminished variability in the amplification reactions using the competitor in solution (page 725, second paragraph). It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used tRNA of Miyamura et al. as a carrier in the formation of plates of Klatser et al. The motivation to do so, provided by Miyamura et al., would have been that the presence of tRNA was advantageous because it provided an indicator of RNA degradation (col. 3, lines 4-9). 16.

Applicant respectfully disagrees in view of the above response to points 11 and 12 of the Office Action.

The reference Mijamura reports the addition of carrier RNA to serum for use together with HCV detection with the background to improve the stability of complete virus particles in clinical samples. This application appears to have no relation to the present invention because no stabilization of standard nucleic acids is taught by the reference Mijamura.

Applicant concludes that the art rejections of the office Action do not hold water in view of the coated reaction chambers of the present application.

Although some of the individual steps required according to the caims of the present application, within the process to manufacture "reaction chambers coated with nucleic acids" are known in different references, there is no teaching or suggestion of the present invention derivable from these references..

It is the opinion of the applicant that reaction chambers coated with calibrated standard nucleic acids with addition of carrier nucleic acids are novel and unobvious relative to the state of the art. The reaction chambers are also non-obvious since the quality of the standard nucleic acids is decisively improved by the coating in the reaction chambers and this improvement of the quality of the standard nucleic acids was unexpected even by the combination of the references applied. The improved stability is described in the description of the present application and can be gathered in particular from the drawings.

Reconsideration of all outstanding rejections is respectfully requested.

Entry of the present amendment is respectfully requested. All claims as presently submitted are deemed to be in form for allowance and an early notice of allowance is earnestly solicited.

Respectfully submitted,
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